

In the Claims

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing.

1. (Original) A method for determining an amino acid sequence binding motif for a phosphorylation site of a kinase, comprising:

a) contacting the kinase with a peptide library, wherein each peptide comprises a single non-degenerate phosphorylatable amino acid in a fixed position of the peptide and wherein each peptide comprises one or more degenerate amino acids, under conditions which allow for binding of a peptide by the kinase at the phosphorylation site of the kinase;

b) allowing the kinase to bind peptides of the peptide library having a binding site for the kinase phosphorylation site to form kinase-peptide complexes;

c) isolating the kinase-peptide complexes from the unbound peptides;

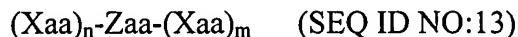
d) releasing the peptides from the kinase-peptide complexes;

e) isolating the peptides previously bound to the kinase in kinase-peptide complexes;

f) determining the amino acid sequences of the peptides isolated in (e); and

g) determining an amino acid sequence motif for a binding site of the kinase based upon the relative abundance of different amino acid residues at each degenerate position within the peptides.

2. (Original) The method of claim 1, wherein the peptide library comprises peptides comprising the formula:



wherein Zaa is the single non-degenerate phosphorylatable amino acid and is selected from the group consisting of Tyr, Ser and Thr,

wherein Xaa is any amino acid except Zaa, and

wherein n and m are integers from 1-10 inclusive.

3. (Original) The method of claim 1, wherein the peptide library is a soluble synthetic peptide library.

4. (Original) The method of claim 1, wherein the single non-degenerate phosphorylatable amino acid is tyrosine.

5-8. (Canceled)

9. (Original) The method of claim 1, wherein the single non-degenerate phosphorylatable amino acid is serine.

10-12. (Canceled)

13. (Original) The method of claim 1, wherein the single non-degenerate phosphorylatable amino acid is threonine.

14-65. (Canceled)

66. (New) The method of claim 4, wherein the peptides comprise the amino acid sequence Xaa_n -Tyr- Xaa_m (SEQ ID NO:14), wherein Xaa is any amino acid except Tyr and wherein n and m are integers from 1-10 inclusive.

67. (New) The method of claim 4, wherein the peptides comprise the amino acid sequence Xaa_n -Tyr- Xaa_m (SEQ ID NO:15), wherein Xaa is any amino acid except Tyr or Cys and wherein n and m are integers from 1-10 inclusive.

68. (New) The method of claim 4, wherein the peptides comprise the amino acid sequence Xaa_n -Tyr- Xaa_m (SEQ ID NO:16), wherein Xaa is any amino acid except Tyr, Cys or Trp and wherein n and m are integers from 1-10 inclusive.

69. (New) The method of claim 7, wherein the peptides comprise the amino acid sequence Xaa₄-Tyr-Xaa₄ (SEQ ID NO:17), wherein Xaa is any amino acid except Tyr, Cys or Trp .
70. (New) The method of claim 9, wherein the peptides comprise the amino acid sequence Xaa_n-Ser-Xaa_m (SEQ ID NO:18), wherein Xaa is any amino acid except Ser and wherein n and m are integers from 1-10 inclusive.
71. (New) The method of claim 9, wherein the peptides comprise the amino acid sequence Xaa_n-Ser-Xaa_m (SEQ ID NO:19), wherein Xaa is any amino acid except Ser or Cys and wherein n and m are integers from 1-10 inclusive.
72. (New) The method of claim 9, wherein the peptides comprise the amino acid sequence Xaa_n-Ser-Xaa_m (SEQ ID NO:20), wherein Xaa is any amino acid except Ser, Cys or Trp and wherein n and m are integers from 1-10 inclusive.
73. (New) The method of claim 13, wherein the peptides comprise the amino acid sequence Xaa_n-Thr-Xaa_m (SEQ ID NO:21), wherein Xaa is any amino acid except Thr and wherein n and m are integers from 1-10 inclusive.
74. (New) The method of claim 13, wherein the peptides comprise the amino acid sequence Xaa_n-Thr-Xaa_m (SEQ ID NO:22), wherein Xaa is any amino acid except Thr or Cys and wherein n and m are integers from 1-10 inclusive.
75. (New) The method of claim 13, wherein the peptides comprise the amino acid sequence Xaa_n-Thr-Xaa_m (SEQ ID NO:23), wherein Xaa is any amino acid except Thr, Cys or Trp and wherein n and m are integers from 1-10 inclusive.
76. (New) The method of claim 1, wherein the peptide library is contacted with the kinase by application of the library to a substrate to which the kinase is immobilized.

77. (New) The method of claim 1, wherein the kinase-peptide complexes are isolated by washing the kinase-peptide complexes in a buffer that permits binding of peptides to the phosphorylation site of the kinase.

78. (New) The method of claim 1, wherein the peptides are eluted from the kinase-peptide complexes by incubating the kinase-peptide complexes with an elution solution.

79. (New) The method of claim 78, wherein the elution solution has an acidic pH.